Pathogenic Fungi Occurring on the Noogoora Burr Complex in Australia

LOUISE MORIN, BRUCE A. AULD, JOHN F. BROWN and M. ABDUL CHOLIL

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The Noogoora burr (cocklebur) complex in Australia consists of four closely related *Xanthium* species considered to be among the most important weeds in summer crops and pasture. In 1987, a mail survey was sent to agronomists to determine the distribution of the Noogoora burr complex and the occurrence of rust, *Puccinia xanthii*, on *Xanthium* spp. (other than *X. spinosum*) in Australia. Field surveys were made in late summer of 1985 and 1990 in New South Wales and Queensland, and during April 1991 in the Northern Territory and the north of Western Australia to supplement results obtained from the mail survey. *Puccinia xanthii* was most prevalent and severe in south-east Queensland but also occurred in the other States surveyed. In Queensland, leaf, petiole and stem lesions were common and adversely affected growth of plants. During the field surveys of 1990 and 1991, attempts were made to isolate other pathogenic fungi occurring on *Xanthium* spp. Several soil-borne pathogens were isolated from stems of wilting plants. Other fungi including species of *Phomopsis*, *Colletotrichum* and *Alternaria* were isolated from diseased foliage. The pathogenicity of 63% of the fungi isolated was assessed to evaluate their potential as mycoherbicides. *Alternaria zinniae* and *P. xanthii* were the most aggressive pathogens but the latter is not a suitable candidate for development as a mycoherbicide because of its biotrophic nature. A close association between some facultative parasites and rust was observed in the field.

L. Morin, Agriculture Canada, P.O. Box 440, Regina, SK, Canada, S4P, 3A2. B. A. Auld, NSW Agriculture, Agricultural Research & Veterinary Centre, Forest Road, Orange, NSW 2800, Australia, and J. F. Brown, M. A. Cholil, Department of Botany, University of New England, Armidale, NSW 2351, Australia; present address of M. A. Cholil, Departmen Hama and Penyakit, Facultas Portanian, Universitas Brawijaya, Malang, Indonesia; *manuscript received 9 February 1993, accepted for publication 21 April 1993.*

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INTRODUCTION

The genus *Xanthium* in Australia comprises five robust annual weed species, *X. spinosum* L. (Bathurst burr, spiny cocklebur) and other *Xanthium* spp. (Noogoora burr [cocklebur] complex) which cause major economic losses in agricultural production (Martin and Carnahan, 1983; Hocking and Liddle, 1986). The recognition of four different but closely-related species within the Noogoora burr complex in Australia is widely accepted (Hocking and Liddle, 1986). The complex consists of *X. occidentale* Bertol. (Noogoora burr), *X. italicum* Mor. (Hunter burr), *X. orientale* L. (Californian burr) and *X. cavanillesii* Schouw (South American burr). These species are commonly grouped under the name *X. strumarium* L. *sensu lato* in the literature. *Xanthium* seedlings are poisonous to livestock, growing plants compete with summer crops and pasture and the spiny fruits characteristic of the genus contaminate the wool of sheep. Weeds of the Noogoora burr complex are also hosts for parasitic *Cuscuta* spp., major pests of lucerne (*Medicago sativa* L.).

Xanthium spp. are susceptible to some pre- and post-emergence broad-leaf herbicides but chemical control in Australia is generally uneconomical because most infestations occur on vast pastoral areas. Chemical control also is not feasible when the weeds grow in crops such as cotton (*Gossypium hirsutum* L.) and sunflower (*Helianthus annuus* L.) which are susceptible to the available herbicides (Hocking and Liddle, 1986). Hand-hoeing of these species is still practised in cotton in Australia. Biological control of

Xanthium weeds with fungi represents a possible alternative or complementary strategy to traditional control measures.

An anthracnose fungus, *Colletotrichum orbiculare* (Berk. & Mont.) v. Arx is being developed as a mycoherbicide to control *X. spinosum* L. (Auld *et al.*, 1988; McRae and Auld, 1988; Auld *et al.*, 1990). This fungus, however, is ineffective as a biological control agent for the Noogoora burr complex (Nikandrow *et al.*, 1990; B. A. Auld, unpublished data). Pathogenic fungi including a species of *Phomopsis*, *Verticillium dahliae* Klebahn and *Macrophomina phaseolina* (Tassi) Goidanich were isolated from diseased *Xanthium* spp. belonging to the Noogoora burr complex during the late summer of 1984 in New South Wales (Nikandrow *et al.*, 1990). None of these fungi, however, demonstrated great potential for use as biological control agents because of their low virulence or wide host range. The rust fungus *Puccinia xanthii* Schw. has been proposed as a potential classical biological control agent for the Noogoora burr complex in Australia (Hasan, 1974). It was first recorded in Australia near Brisbane, Queensland in 1975 and has since spread throughout Queensland, New South Wales (NSW) and the Northern Territory (Alcorn, 1975; Alcorn and Kochman, 1976; Hocking and Liddle, 1986). The epidemiology of *P. xanthii* and extent of damage it causes on the Noogoora burr complex have been investigated recently (Morin *et al.*, 1992, 1993) but the distribution of the disease is not well documented.

The present study was undertaken to determine the occurrence of rust and other pathogenic fungi on the Noogoora burr complex in Australia. The potential for use of these fungi as inundative or classical biological control agents is assessed.

Materials and Methods

Mail survey — occurrence of the rust

In early 1987 a mail survey was distributed to 105 agronomists in Queensland, NSW and Victoria to determine the occurrence of *P. xanthii* on *Xanthium* spp. The survey consisted of an introductory letter and pictorial identification guides to *Xanthium* spp. and rust symptoms as well as a questionnaire. The latter requested information about the occurrence of *Xanthium* spp. present in a particular area and the occurrence of rust on the respective species. Similar information was obtained for Western Australia, the Northern Territory and Tasmania by contacting relevant weed agronomists.

Field surveys — occurrence of the rust

Field surveys were made during the late summer months of 1985 and 1990 in NSW and Queensland to supplement the results obtained from the mail survey. In addition, three sites in South Australia and Victoria infested with species of the Noogoora burr complex were examined in 1985 for presence of rust infection. A subsequent field survey in 1991 targeted the main areas of the Northern Territory and Western Australia infested with *Xanthium* spp. to determine the occurrence and severity of rust in northern regions of Australia. The sites inspected were generally located on roadsides, creek and river banks, and paddocks.

Collection and isolation of fungi from Xanthium spp.

During the field surveys undertaken in late summers 1990 and 1991, plants of the Noogoora burr complex showing disease symptoms that differed from those resulting from rust infection were collected. Sixty-five collections of diseased *Xanthium* plants were made at 37 different sites infested with one or two of the species of *X. occidentale*, *X. italicum* and *X. cavanillesii*. Leaf tissue with disease symptoms was cut into small pieces (approximately 0.5 cm^2), surface sterilized in 2% sodium hypochlorite for 1 to 3 min

and transferred to Petri dishes containing low-strength potato dextrose agar (4 g PDA, 15 g agar, 1 L pure H_2O) (filtered by a double-pass reverse osmosis water purification system, model Milli-RO TS® , Millipore S.A., Molsheim, France) acidified with lactic acid (one drop of 25% lactic acid per plate). Diseased stems, petioles and fruits were surface sterilized in 70% ethanol for 30 sec and in 2% sodium hypochlorite for 1 to 6 min, cut into small pieces and transferred to acidified low-strength PDA contained in Petri dishes. The plates were incubated in the dark at 25° ± 1°C for approximately one month. Fungi growing from the pieces of plant tissue were sub-cultured onto PDA and low-strength PDA contained in Petri dishes. Plates were incubated in the dark at 25°C until sporulation was observed. Fungal spores produced on these plates or directly on the plant tissue were transferred using a sterile inoculating loop to acidified low-strength PDA and streaked across the surface of the agar. Plates were incubated in the dark at 25°C for 18 to 24 h before single germinating spores were transferred onto PDA and low-strength PDA using a fine sterile needle to make a single-spore isolate. Mycelial isolates were made from the fungi that did not sporulate in artificial culture. Single-spore and mycelial isolates of fungi isolated from diseased specimens were preserved in McCartney bottles in saline solution (Muir, 1988) and on low-strength PDA slopes and stored in a cold room at 4°C (Agricultural Research & Veterinary Centre, Orange, Australia). Spores produced by most single-spore isolates were also preserved in a skim-milk and glycerol solution (D. TeBeest, personal communication) in a cryofreezer (Forma Scientific, model 8471, Ohio, USA) at -70°C.

Inoculum production and preliminary screening tests for pathogenicity

A preliminary experiment was performed in controlled environment conditions to screen the numerous single-spore fungal accessions for pathogenicity on *X. occidentale*. Time and space restrictions limited the number of plants that could be inoculated. Soil-pathogens including *Fusarium* spp., *M. phaseolina*, *V. dahliae* and *Sclerotinia sclerotiorum* (Lib.) de Bary which usually have a wide host range, yeast-like fungi and isolates that did not sporulate abundantly on artificial media after two months were not included in the preliminary pathogenicity tests.

Inoculum of the Hyphomycete fungi was produced by transferring small pieces of mycelium from the single-spore colony maintained on agar slopes to oatmeal agar (OMA, 20 g oatmeal, 20 g agar, 1 L pure H_2O), PDA and low-strength PDA in Petri dishes. Plates were placed in small plastic bags in an incubator set at 25°C with a 12 h photoperiod consisting of near ultra-violet (NUV) (Splendor, 20W) and white (Osram, 20W) lights. Twenty days after inoculation of plates, spores were harvested from the medium that produced maximum sporulation either with a cyclone spore collector (Tervet and Cherry, 1950) and subsequently suspended in water or by flooding the plates with a solution of pure water and Tween 80® (approximately two to three drops Tween 80® per 100 mL H_2O). The latter suspension was then filtered through cheesecloth to separate the spores from the mycelial fragments. The spore density of the suspensions was determined using a haemocytometer and adjusted to densities of 1×10^4, 1×10^5 or 5×10^5 spores/mL depending on the number of spores present, using pure water.

Inoculum of non-Hyphomycete fungi was produced by transferring spores with a sterile needle, from the original single-spore colony to a sterile glass slide containing a drop of sterile pure water to make a spore suspension. Petri dishes containing OMA, PDA and low-strength PDA were inoculated by streaking the surface of the agar with the spore suspension using a sterile inoculating loop. One mL of sterile pure water was then added to each plate and spread evenly onto the surface with a sterile bent glass rod. Plates were placed in small plastic bags and incubated at 25°C with a 12 h NUV and

white lights/12 h dark regime. Plates were examined every second day for sporulation. Spores were harvested from the medium that produced maximum sporulation seven days (± two days) after half of the fungal colony was observed to be sporulating. The surface of the agar was flooded with approximately 5 mL of pure water and gently flushed several times using a 5 mL disposable syringe which was then used to recover the spores. The suspension was transferred to a McCartney bottle, spore density determined with a haemocytometer and adjusted to 1 x 10^6 spores/mL using pure water.

Two Noogoora burr plants at the four- to five-leaf stage grown in a sand-peat mixture contained in 10-cm diameter plastic pots in a temperature-controlled glasshouse at 25° ± 1°C were sprayed with the spore suspensions until run-off using an N_2-propelled spray atomizer (Badger air-brush co., model no. 250-1, Illinois, USA) for each fungal accession. Inoculated plants were placed in a dark dew chamber (Percival, model I-60 DL, Boone, Iowa) set at 24° ± 1°C for 24 h and then transferred to a walk-in controlled environment chamber (Conviron, model PGV 36, Winnipeg, Man., Canada) set at 25° ± 1°C, 55% RH and 12 h photoperiod (330 $\mu Em^{-2}s^{-1}$). Plants were examined for disease symptoms at one and two weeks after inoculation. One plant was then transferred to a dark controlled environment chamber (Conviron, model E7H) set at 25° ± 1°C for two weeks to stimulate rapid senescence. Both plants were examined again for disease symptoms at four weeks after inoculation.

The fungi were re-isolated from diseased plant tissue, to conform with Koch's postulates, using the isolation technique employed for leaf specimens collected from the field. Attempts were also made to re-isolate the fungi from healthy plant tissue of the third oldest leaf of inoculated plants.

Pathogenicity test

Fungi that produced disease symptoms on Noogoora burr plants in the preliminary pathogenicity test were further tested to confirm their pathogenicity. Inocula of the fungi (except *Alternaria zinniae* M. B. Ellis; IMI352084, DAR68414) were produced by spreading with a sterile inoculating loop approximately 0.5 mL of a thawed suspension of spores in a skim-milk and glycerol solution onto the surface of PDA contained in Petri dishes. Plates were placed in small plastic bags in an incubator set at 25°C with a 12 h NUV and white lights/12 h dark regime. Inoculum of *A. zinniae* was produced with a method slightly modified from Nehl (1990). At one month after inoculation of plates, spores of all fungi were collected by washing the surface of the agar with pure water. The density of spore suspensions was determined with a haemocytometer and adjusted to 1 x 10^6 spores/mL (1 x 10^4 spores/mL for *A. zinniae*) using pure water.

Noogoora burr plants at the two- to three-leaf stage were sprayed with the spore suspensions as described above. Control plants were sprayed with water. Inoculated and control plants were placed in a dark dew chamber at 24°C for 24 h and then transferred to a walk-in controlled environment chamber set at 20° ± 1°C, 55% RH and 12 h photoperiod (580 $\mu Em^{-2}s^{-1}$). Disease symptoms, if any, were recorded at one week after inoculation before the lights in the controlled environment chamber were turned off. Plants were left in the dark for 20 days to stimulate senescence. The surface of senescent plants was then examined for fruiting bodies of the fungi. Fungi were re-isolated from the part of the stem between the second and third oldest leaves using the procedure described above. A completely randomized design with six replicates per treatment was used in this experiment which was performed twice.

Results

Distribution of the Noogoora burr complex

X. occidentale was the most abundant species of the Noogoora burr complex occurring in Australia (Fig. 1). It was very widespread in Queensland and New South

Wales during the mail (1987) and field surveys (1985, 1990). It also occurred in the northern and central regions of the Northern Territory. Infestations around Darwin were not observed in 1991 because they had been eradicated with herbicides (I. Miller, personal communication). In the north of Western Australia, Noogoora burr infested large areas along the Ord River (1991). These areas are currently under quarantine to restrict the spread of the weed throughout the state. Infestations of Noogoora burr in Western Australia were also reported on the Fitzroy River and in a few areas around Perth. Populations of Noogoora burr were reported in the south-eastern part of South Australia and in Victoria but the distribution of the weed was difficult to evaluate since only a few sites were visited.

Fig. 1. Distribution of the Noogoora burr complex in Australia according to mail and field surveys: *Xanthium occidentale* (•), *X. orientale*, *X. italicum*, *X. cavanillesii* and putative hybrid between *X. italicum* and *X. occidentale* (△).

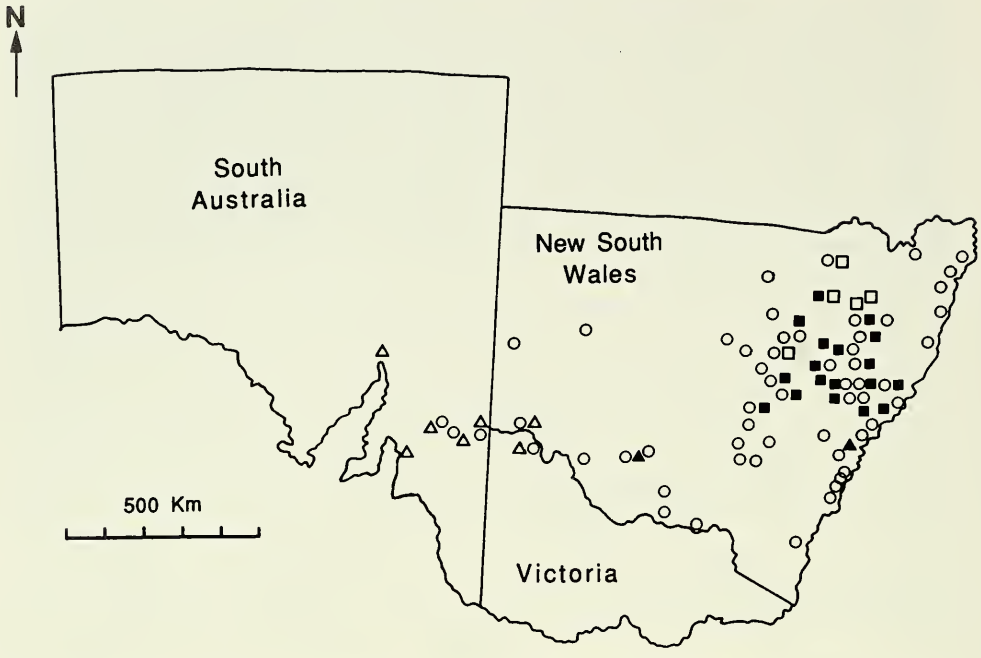

Fig. 2. Detailed distribution of the Noogoora burr complex in New South Wales, Victoria and South Australia according to mail and field surveys: *Xanthium occidentale* (•), *X. orientale* (△), *X. italicum* (■), *X. cavanillesii* (▲) and putative hybrid between *X. italicum* and *X. occidentale* (□).

The other *Xanthium* spp. included in the Noogoora burr complex were not widely distributed in the regions of Australia surveyed (Fig. 2). The results from the mail and field surveys indicated that *X. italicum* and *X. orientale* occurred on approximately 17% and less than 5%, respectively, of the total areas infested with the Noogoora burr complex. The collection of putative hybrids between *X. occidentale* and *X. italicum* at 19% of the sites visited during the 1990 and 1991 field surveys indicated the presence of hybridizing mixed populations of these species. Pure and hybridized stands of *X. italicum* were mainly reported in areas of central and north-eastern NSW and along the Hunter river. In contrast, infestations of *X. orientale* were found in South Australia and in Victoria. Small populations of *X. cavanillesii* were found only at Richmond (near Sydney) and on the bank of the Murrumbidgee River near Darlington Point, NSW. No infestations of the Noogoora burr complex were recorded in Tasmania.

The field surveys indicated that more than 50% of the infestations of the Noogoora burr complex occurred on the banks of rivers and creeks. Infestations were less extensive along road sides and cultivated paddocks.

Occurrence of the rust

Puccinia xanthii was observed on all species of the Noogoora burr complex during the mail and field surveys, occurring in 50 to 64% of the areas infested with the weeds. The disease was most prevalent and severe in south-eastern Queensland and north-eastern NSW (Fig. 3). Rust was reported from all States except Victoria and Tasmania. Only one site infested with *Xanthium* spp., however, was inspected for the occurrence of rust in Victoria. In 1991, epidemics of the rust were not severe in the north of Western Australia and Northern Territory. *P. xanthii* was reported at two sites in South Australia during

the mail survey. During the 1990 and 1991 field surveys, leaf lesions were common symptoms in all populations of *Xanthium* spp. infected by the rust. In Queensland, however, leaf, petiole and stem lesions were frequently observed simultaneously and appeared to adversely affect growth of plants.

Fig. 3. Distribution of the rust *Puccinia xanthii* in Australia according to mail and field surveys during the period 1985-1991.

Collection and isolation of fungi from Xanthium spp.

In 1990 and 1991 a total of 164 fungal accessions were isolated from plants showing disease symptoms (45 collections) collected from 27 sites in NSW, Queensland, Northern Territory and West Australia (Table 1). Six *Colletotrichum* spp. were isolated from the Noogoora burr complex: *C. acutatum* Simmonds ex Simmonds (DAR63299a), *C. coccodes* (Wallr.) S. J. Hughes (DAR63322), *C. dematium* (Pers.: Fr.) Grove (type A: DAR63299c, type B: DAR67501), *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz. *sens.*

TABLE 1

Fungi isolated from Xanthium occidentale *and* Xanthium italicum *during the 1990 and 1991 field surveys in New South Wales and Queensland, and Northern Territory and Western Australia, respectively*

Fungus	Isolation frequency from[p]							Preliminary pathogenicity test[s]	Re-isolation of fungi[t]
	X. occidentale			*X. italicum*		Hybrid[q]			
	L[r]	S	F	L	S	L	S		
Colletotrichum acutatum DAR63299a[u]	-[v]	4	–	–	1	–	1	6	5
C. dematium (type A)[w] DAR63299c	5	1	–	–	–	–	1	7	7
C. dematium (type B) DAR67501	–	1	–	–	–	–	–	1	1
C. coccodes DAR63322	–	2	–	–	–	–	–	1	1
C. gloeosporioides (type A)[w] DAR67499	5	3	–	1	–	–	–	9	9
C. gloeosporioides (type B) DAR69289	–	3	–	–	–	–	–	3	3
C. orbiculare DAR63302b	–	1	–	–	–	–	–	1	1
C. truncatum DAR67500	1	–	–	–	–	–	–	1	0
Glomerella cingulata[x] DAR67498	3	1	–	–	–	–	–	0	–
Phomopsis sp. DAR69288	1	5	–	3	–	–	2	9	5
Diaporthe arctii DAR65715	–	1	–	–	–	–	–	1	0
Alternaria zinniae DAR68414	–	1	–	–	–	–	–	1	-[y]
Sclerotinia sclerotiorum DAR65690	–	–	–	–	–	–	1	0	–
Macrophomina phaseolina DAR69498	–	2	–	–	–	–	–	0	–
Verticillium dahliae DAR69497	–	1	–	–	–	–	–	0	–
Phaeotrichoconis crotolariae (Salam & Rao) Subram. DAR67497	1	–	–	–	–	–	–	0	–
Plectosphaerella cucumeris Klebahn[z] DAR65688	–	1	–	–	–	–	–	1	1
Fusarium spp.	2	12	–	2	2	–	8	0	–
Pestalotiopsis sp.	2	1	1	–	–	–	–	4	4
other Hyphomycetes	6	9	–	2	2	1	6	20	13
other Coelomycetes	2	25	1	6	1	–	9	36	7
yeast-like	3	1	–	1	–	–	–	0	–
other Ascomycotina	2	3	–	–	–	–	1	2	0
Total	33	78	2	15	6	1	29	104	57

[p] Number of fungal accessions isolated from different collections of diseased plants and belonging to the same subdivision, class, genus or species.

[q] Putative hybrid between *X. occidentale* and *X. italicum*.

[r] Organ of plants showing disease symptoms from which the fungi were originally isolated: L=leaf, S=stem, F=fruit.

[s] Number of fungal accessions preliminary tested for pathogenicity on *X. occidentale*.

[t] Number of fungal accessions re-isolated from apparently non-diseased tissue of inoculated *X. occidentale* plants.
[u] Accession number of fungi located at the Biological and Chemical Research Institute, Rydalmere, NSW, Australia.
[v] Not applicable.
[w] Two types of *C. gloeosporioides sens. lat.* and *C. dematium* with different cultural characteristics were isolated.
[x] The anamorph (*C. gloeosporioides*) of the accessions were not observed in artificial culture.
[y] Inoculated plants were severely diseased and therefore the fungi were not isolated from apparently non-diseased tissue.
[z] Teleomorph of *Fusarium tabacinum* (Beyma) W. Gams.

lat. (type A: DAR67499, type B: DAR69289) (teleomorph: *Glomerella cingulata* (Stonem.) Spauld. & Schrenk., DAR67498), *C. orbiculare* (DAR63302b), and *C. truncatum* (Schw.) Andrus & Moore (DAR67500). The species *C. acutatum* and *C. gloeosporioides* were the only ones recorded on *X. italicum* and none were recorded on *X. cavanillesii. C. acutatum, C. dematium* and *C. orbiculare* were found in association with *P. xanthii* on *X. occidentale. C. dematium* and *C. acutatum*, however, were also isolated from plants growing in rust-free sites. It was common to observe salmon-coloured spore masses of *Colletotrichum* spp. on the surface of black swollen stem lesions in the field in Queensland. In most cases, teliospores of *P. xanthii* were found inside the swollen part of the stem. These surveys indicated that *C. gloeosporioides sens. lat.* was the most prevalent species on *Xanthium* spp. followed by *C. dematium* and *C. acutatum. C. orbiculare* was isolated on one occasion in Queensland from a Noogoora burr plant growing among a population of Bathurst burr infected with the same fungus. The prevalence of *Colletotrichum* spp. was higher in Queensland than in NSW.

A species of *Phomopsis* was consistently isolated from small leaf lesions or brown elongated stem lesions (Table 1). The teleomorph of one species of *Phomopsis* isolated from Queensland was identified as being closely related to *Diaporthe arctii* (Lasch) Nitschke (DAR65715). Soil-borne fungal pathogens including *M. phaseolina* (DAR69498) and *V. dahliae* (DAR69497) were isolated from the stems of wilting plants (Table 1). *Sclerotinia sclerotiorum* (DAR65690) was isolated and believed to be responsible for severe wilting in a dense mixed hybridizing population of *X. occidentale* and *X. italicum* in NSW. Several *Fusarium* spp. were particularly abundant on stems of diseased *Xanthium* plants growing in north-eastern NSW and south-eastern Queensland. *Alternaria zinniae* was found in association with *P. xanthii*, *Colletotrichum* spp. and *Phomopsis* sp. on a stem lesion of *X. occidentale* in Queensland. Other fungi belonging to the Deuteromycotina and Ascomycotina were isolated but not identified further because they did not produce any disease symptoms during the preliminary screening tests for pathogenicity.

Preliminary screening tests for pathogenicity

In preliminary screening tests, the pathogenicity of 63% of fungi isolated from plants of the Noogoora burr complex was assessed (Table 1). Twelve percent of the fungal accessions tested for pathogenicity produced disease symptoms on Noogoora burr (Table 2). Fifty-five percent of isolates tested were re-isolated from apparently non-diseased plant tissue at four weeks after inoculation. The accession of *A. zinniae* was the most aggressive pathogen on Noogoora burr. Plants inoculated with a spore suspension of *A. zinniae* and exposed to a 24 h dew period at 24°C developed necrotic leaf lesions (Table 2). None of the other fungal accessions inoculated onto Noogoora burr plants showed such a high level of virulence. Mild symptoms in the form of a few chlorotic or necrotic flecks were observed on most plants inoculated with *C. acutatum, C. gloeosporioides* type B and *C. orbiculare* at one week after inoculation. Among the accessions of *Colletotrichum* tested, only one of *C. acutatum* (isolate no. 4) was not re-

isolated from diseased plant tissue at four weeks after inoculation (Table 2). Sunken, watery stem lesions developed on plants inoculated with two accessions of *C. gloeosporioides* type B and *Phomopsis* sp. two weeks after inoculated plants were transferred to a dark controlled environment chamber.

TABLE 2

Fungal accessions apart from Puccinia xanthii *that produced disease symptoms on inoculated* Xanthium occidentale *plants in preliminary pathogenicity tests and were subsequently extensively tested for pathogenicity*

Fungi	AR & VC accession no.[t]	DAR/IMI accession no.[u]	Preliminary pathogenicity test: Disease symptoms[v]	Pathogenicity test[s]: Disease symptoms[w]	Presence of fungal fruiting bodies[x] (%)	Re-isolation of fungi[y] (%)
Colletotrichum acutatum	1	DAR63299a	F	CF	75	100
	4		F	CF	42	100
	9		F	CF	92	100
	71		F	CF	92	100
	73		F	CF	75	100
	74		F	CF	67	100
C. gloeosporioides	8	DAR63321[z]	F	—	25	92
	43	DAR69289	S	NF	92	100
	183	DAR69499	S	—	92	100
C. orbiculare	50	DAR63302b	F	NF	67	100
Alternaria zinniae	76	DAR68414 IMI352084	L	L	0	100
Phomopsis sp.	61		S	—	0	100
	140		S	—	0	92

[s] Results are from pooled experiments

[t] Accession number of fungi kept at the Agricultural Research & Veterinary Centre, Orange, NSW, Australia.

[u] Accession number of fungi lodged at the Biological & Chemical Research Institute, Rydalmere, NSW, Australia (DAR) or at the C.A.B. International Mycological Institute, Surrey, UK (IMI).

[v] F = flecks observed on leaves at 1 week after inoculation; S = stem lesion observed at 4 weeks after inoculation on plants subjected to a dark period for the last 2 weeks before disease assessment; L = necrotic lesions observed at 1 week after inoculation. All accessions except *C. acutatum* accession no. 4 were re-isolated from diseased plant tissue at 4 weeks after inoculation.

[w] Disease symptoms observed on leaves of inoculated plants at 1 week after inoculation: — = no visible disease symptoms; CF = numerous chlorotic flecks; NF = few necrotic flecks; L = necrotic leaf lesions.

[x] Percentage of inoculated plants on which fruiting bodies of the fungi were observed *in situ* on senescent stem tissue.

[y] Percentage of inoculated plants from which fungi were re-isolated from the stem.

[z] Identified as close to *C. destructivum* O'Gara based on the character of a few spores being 'boat shaped'.

Pathogenicity test

Pathogenicity of the fungi that produced disease symptoms in preliminary pathogenicity tests was confirmed in a subsequent extensive experiment (Table 2). All accessions of *C. acutatum* produced numerous chlorotic flecks on leaves of inoculated plants in less than one week after inoculation. *C. orbiculare* and one accession of *C. gloeosporioides* type B only produced few necrotic flecks on leaves. *Alternaria zinniae* produced severe disease symptoms on plants inoculated with a low density suspension of spores. No visible disease symptoms were observed at one week after inoculation on

plants inoculated with the accessions of *Phomopsis* sp. and with two of the accessions of *C. gloeosporioides* type B. All accessions except *A. zinniae* and *Phomopsis* sp. developed fruiting bodies on senescent stems of inoculated plants at the end of the experiment. All fungi were re-isolated from senescent tissue.

Discussion

Distribution of the Noogoora burr complex in Australia as indicated by the mail and field surveys reported in this study differs slightly from that reported by Hocking and Liddle (1986). The infestation of *X. italicum* has now expanded in the region north-east of the Hunter valley, NSW. Furthermore, a small population of *X. cavanillesii* was discovered during the 1990 field survey on the bank of the Murrumbidgee River, where it had not been reported previously. Putative hybrids between *X. italicum* and *X. occidentale* were common in mixed populations of these species in NSW (McMillan, 1975). The most common habitat for species of the Noogoora burr complex was on river and creek banks and flood plains (Martin and Carnahan, 1983).

The rust, *P. xanthii*, was observed to infect all species of the Noogoora burr complex. The occurrence of rust on *X. cavanillesii* reported here is the first record of this biotrophic parasite on this species in Australia (J. Walker, personal communication). The susceptibility of each *Xanthium* spp. to infection by *P. xanthii* was found to be similar under controlled environment conditions (Morin *et al.*, 1993).

Puccinia xanthii has spread considerably since it was first recorded in Australia in February 1975 in Queensland (Alcorn, 1975). In a relatively short period following this, the rust was reported in NSW at Murwillumbah in April 1975, at Moree in May 1976 and at Dareton in March 1979 (M. Priest, personal communication). According to the surveys presented in this paper, rust was observed in most areas infested with the Noogoora burr complex but was not recorded in the small populations of *X. occidentale* in the vicinity of Perth and Fitzroy Crossing, Western Australia and at Alice Springs, Northern Territory. In the north of the Northern Territory, spread and annual epidemics of rust have been enhanced by several deliberate introductions of infected plant parts at the Daly and Victoria River areas (Hocking and Liddle 1986; J. Pitt and I. Miller, personal communication). In 1991, however, no rust infections were observed in these areas. The tropical climate of northern Australia, characterized by high temperatures or low moisture conditions, may explain why the *Xanthium* rust was not well established in these areas. *Puccinia xanthii* depends on a relatively specific combination of climatic conditions to cause epidemics on *Xanthium* weeds (Morin *et al.*, 1992).

Xanthium rust is disseminated by heavy winds and floods as dry leaf fragments covered with telia or dead pieces of stem containing internal telia scattered through the cortical tissue (Hasan, 1974). Alcorn (1975) indicated that dissemination of *Xanthium* rust by natural means may be slower than that of rusts that produce airborne urediniospores or teliospores since teliospores of *P. xanthii* remain attached to the telia during the entire life-cycle. The short-lived and fragile basidiospores produced by the teliospores are not considered to play an important role in dissemination of the pathogen over large areas. Basidiospores of *Puccinia recondita* Roberge ex Desmaz., for example, may only be transported by wind over a distance of about 5 meters (Yamada *et al.*, 1973).

Hocking and Liddle (1986) commented that rust had not provided significant large-scale control of the Noogoora burr complex since its introduction into Australia. The severe damage observed on rust-infected plants in Queensland during the field surveys suggested that *P. xanthii* was effective in controlling these. Since the mid 1960's a large reduction in Noogoora burr populations in the eastern high-rainfall regions of Queensland has been linked to the presence of rust (Chippendale, 1993). Julien *et al.*

(1979) demonstrated that *P. xanthii* affects the fitness of *Xanthium* weeds by shortening the plant life cycle, reducing plant growth rate and seed production. The effect of pathogens on the fitness of weedy plants is believed to play an important role in altering the size of weed populations, thereby creating new potential niches for other less dominant plant species and consequently affecting whole plant communities (Burdon, 1991). The rust *Puccinia lagenophorae* Cooke, for example, has been observed to reduce competitiveness of the weed *Senecio vulgaris* L. with other plant species (Paul and Ayres, 1990). In Australia, the reduction of competitiveness of form A of the weed *Chondrilla juncea* L. in response to infection by the rust *Puccinia chondrillina* Bubak & Syd. (race A) had a considerable impact on the distribution and abundance of rust resistant forms B and C of the weed (Burdon *et al.*, 1981).

The introduction of *Xanthium* rust as a classical biological control agent to new areas infested with the Noogoora burr complex or to assist existing inoculum of *P. xanthii* in areas where its effectiveness is frequently reduced by unfavorable environmental conditions should be considered so as to impose selective pressure on the weed populations. Agronomists, however, should be aware that some cultivars of sunflower are slightly susceptible to *P. xanthii* (Alcorn, 1976; Kochman, 1980; Morin *et al.*, 1993).

The inundative approach, characterized by application of a mycoherbicide, is another potential biological control strategy for the Noogoora burr complex. Deuteromycete fungi have been emphasized in mycoherbicide research because they are readily grown on artificial media. The ideal fungal candidate to be developed as a mycoherbicide should sporulate on artificial media, demonstrate a restricted host range and be highly virulent (Templeton *et al.*, 1979). These characteristics were considered when selecting the fungal accessions obtained from the field surveys for preliminary pathogenicity testing.

The soil-borne wilt-inducing fungi *S. sclerotiorum*, *M. phaseolina*, *V. dahliae* and *Fusarium* spp. were not tested for pathogenicity on *Xanthium* spp. since their high virulence on a wide range of plant species limits their potential as mycoherbicides. Sands *et al.* (1990), however, suggested the possibility of using genetically transformed fungi such as *S. sclerotiorum*, which have lost the ability to sporulate, are auxotrophic or have a reduced host range, as mycoherbicides for broad-leaf weeds. Non-phytopathogenic soil-borne fungi that produce phytotoxic metabolites which stunt or kill weeds are also investigated as potential mycoherbicides (Jones *et al.*, 1988).

The accession of *A. zinniae* produced necrotic leaf and stem lesions when its spores were sprayed onto *X. occidentale* and was the most aggressive pathogen apart from *P. xanthii*. The potential of *A. zinniae* to control the *Xanthium* weeds has been demonstrated by Nehl (1990) but the host range of some accessions of the fungus, which extends to several other plant species, may limit its scope as a mycoherbicide in the field (Auld *et al.*, 1992). Auld *et al.* (1992) emphasized that more field collections of the fungus may provide isolates with a restricted host range since the fungus appeared to be variable.

Several of the fungi isolated from diseased *Xanthium* spp. during the 1990 and 1991 surveys have previously been reported to occur on these weeds. Plants of the Noogoora burr complex are known to be highly susceptible or symptomless-carriers of various fungal pathogens including *Sclerotinia* spp. (Adams *et al.*, 1983; Phillips, 1992), *Verticillium* sp. (Evans, 1968; McKeen and Thorpe, 1973), *Fusarium* spp. (Helbig and Carroll, 1984), *Diaporthe* spp. (*Phomopsis* spp.) (Carrière and Petrov, 1990; Nikandrow *et al.*, 1990) and *Colletotrichum* spp. (Roy, 1982; Hartman *et al.*, 1986; Nikandrow *et al.*, 1990; Walker *et al.*, 1991).

The re-isolation from apparently non-diseased tissue of 54% of the fungal accessions tested in preliminary pathogenicity tests suggests that the *Xanthium* spp. may be clinically symptomless carriers of pathogenic fungi or favour growth of neutral

endophytic or epiphytic fungi. A large number of fungi are known to live either epiphytically or endophytically on or in crop plants (Fisher *et al.*, 1992).

Several species of *Colletotrichum* and a species of *Phomopsis* were isolated from the *Xanthium* weeds during this study. The inability of these fungi to cause severe disease symptoms on actively growing Noogoora burr plants and their re-isolation from lesions that developed on senescing plants or from apparently non-diseased tissue suggests that latent colonization of the plants may occur. Muirhead (1981) indicated that some fungi can undergo a period of latency on plants as inactive hyphae within host tissue or as dormant appressoria. *Colletotrichum* and *Phomopsis* spp. are known to colonize various host plants early in the season and not to produce symptoms until senescence (Cerkauskas, 1988). It is therefore possible that *Xanthium* weeds in Australia are a source of inoculum of pathogenic fungi of economic crop plants as has been shown in Yugoslavia (Carrière and Petrov, 1990), the United States (Roy, 1982; Hartman *et al.*, 1986) and, to a lesser extent already in Australia (Evans, 1968). Host-range testing is necessary to examine this hypothesis further. The small chlorotic or necrotic flecks that developed on the Noogoora burr plants inoculated with spore suspensions of *C. acutatum, C. gloeosporioides* type B or *C. orbiculare* may have been the result of a hypersensitive reaction in the host in response to initial penetration by the pathogen (Tomiyama *et al.*, 1982).

Julien *et al.* (1979) noticed the occurrence of a species of *Colletotrichum* on rust infected *Xanthium* plants in the field but did not report the species involved nor did they investigate the phenomenon. In their study of *Colletotrichum* spp. found on *Xanthium* spp., Walker *et al.* (1990) reported that *C. acutatum* and *C. dematium* were generally accompanied by the rust *P. xanthii*. The close association between these parasites was observed in the specimens collected during the surveys reported here. In addition, *C. orbiculare*, which had only been reported to occur on *X. spinosum* and *X. italicum* (Nikandrow *et al.*, 1990), was also isolated from *X. occidentale* plants infected with rust. The association of *Xanthium* rust with *Colletotrichum* spp. as well as with other fungi isolated from plants of the Noogoora burr complex should be further investigated in an attempt to identify any possible synergistic interactions between the species in terms of the disease symptoms they produce. Such synergism has been reported between *Puccinia lagenophorae* and *Botrytis cinerea* Pers. on the weed *Senecio vulgaris* (Hallett *et al.*, 1990).

The range of pathogens and invertebrate herbivores attacking plants and the possible synergy between them is too often overlooked in weed biological control studies. Ramsell and Paul (1990), for instance observed that grazing by several species of mollusc increased on plants infected by rust fungi. The combined action of *P. xanthii* with secondary plant pathogens and the introduced gall-forming moth, *Epiblema strenuana* Walker (Lep: Gelechiidae) appeared to provide acceptable control of Noogoora burr in south-east Queensland during the summer of 1990. However, the usual humid and warm conditions characteristic of this region do not prevail in much of the area infested with *Xanthium* weeds.

In general, *Xanthium* spp. belonging to the Noogoora burr complex demonstrated a high level of resistance to most fungal diseases. The rust parasite *P. xanthii*, which successfully infected plants, is not a suitable candidate to be developed as a mycoherbicide because of its biotrophic nature. In contrast, *A. zinniae* may have great potential as a mycoherbicide if another highly pathogenic isolate with a restricted host range is found. Additional research should be undertaken to investigate the mechanisms involved in infection of Noogoora burr by these two pathogens. A good understanding of these host-pathogen relationships may lead to the development of mycoherbicides for the control of the Noogoora burr complex.

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References

Adams, P. B., Marose, B. H., and Dutky, E. M., 1983. — Cocklebur: A new host for several *Sclerotinia* species. *Plant Disease* 67: 484-485.

Alcorn, J. L., 1975. — Rust on *Xanthium pungens* in Australia. *Australian Plant Pathological Society Newsletter* 4: 14-15.

Alcorn, J. L., 1976. — Host range of *Puccinia xanthii*. *Transactions of the British Mycological Society* 66: 365-367.

Alcorn, J. L., and Kochman, J. K., 1976. — A field record of *Puccinia xanthii* on sunflower. *Australian Plant Pathological Society Newsletter* 5: 33.

Auld, B. A., McRae, C. F., and Say, M. M., 1988. — Possible control of *Xanthium spinosum* by a fungus. *Agriculture, Ecosystems and Environment* 21: 219-223.

——, Say, M. M., Ridings, H. I., and Andrews, J., 1990. — Field applications of *Colletotrichum orbiculare* to control *Xanthium spinosum*. *Agriculture, Ecosystems and Environment* 32: 315-323.

——, Talbot, H. E., and Radburn, K. B., 1992. — Host range of three isolates of *Alternaria zinniae*, a potential biocontrol agent for *Xanthium* spp. *Plant Protection Quarterly* 7: 114-116.

Burdon, J. J., 1991. — Fungal pathogens as selective forces in plant populations and communities. *Australian Journal of Ecology* 16: 423-432.

——, Groves, R. H., and Cullen, J. M., 1981. — The impact of biological control on the distribution and abundance of *Chondrilla juncea* in south-eastern Australia. *Journal of Applied Ecology* 18: 957-966.

Carriere, J.-B., and Petrov, M., 1990. — *Diaporthe (Phomopsis)* sp., a new pathogen of cocklebur (*Xanthium italicum* Moretti) and of sunflower (*Helianthus annuus* L.) *Helia* 13: 93-106.

Cerkauskas, R. F., 1988. — Latent colonization by *Colletotrichum* spp.: Epidemiological considerations and implications for mycoherbicides. *Canadian Journal of Plant Pathology* 10: 297-310.

Chippendale, J. F., 1993. — The biological control of Noogoora burr, *Xanthium occidentale*, in Queensland: An economic perspective. *In:* Delfosse, E. S., and Scott, R. R. (eds.). *Proceedings of the Eighth International Symposium on Biological Control of Weeds, 1992, Lincoln University, Canterbury, New Zealand.* In press. DSIR/CSIRO, Melbourne, Australia.

Evans, G., 1968. — Infection of *Xanthium pungens* by seedborne *Verticillium dahliae*. *Plant Disease Reporter* 52: 976-978.

Fisher, P. J., Petrini, O., and Lappin Scott, H. M., 1992. — The distribution of some fungal and bacterial endophytes in maize (*Zea mays* L.). *New Phytologist* 122: 299-305.

Hallett, S. G., Paul, N. D., and Ayres, P. G., 1990. — *Botrytis cinerea* kills groundsel (*Senecio vulgaris*) infected by rust (*Puccinia lagenophorae*). *New Phytologist* 114: 105-109.

Hartman, G. L., Manandhar, J. B., and Sinclair, J. B., 1986. — Incidence of *Colletotrichum* spp. on soybeans and weeds in Illinois and pathogenicity of *Colletotrichum truncatum*. *Plant Disease* 70: 780-782.

Hasan, S., 1974. — *Xanthium* rust as a possible biological control agent of Bathurst and Noogoora burrs in Australia. *In:* Wapshere, A. J. (ed.). *Proceedings of the Third International Symposium on Biological Control of Weeds, 1973, Montpellier, France*. pp. 137-140. C.A.B., Farnham Royal, England.

Helbig, J. B., and Carroll, R. B., 1984. — Dicotyledonous weeds as a source of *Fusarium oxysporum* pathogenic on soybean. *Plant Disease* 68: 694-696.

Hocking, P. J., and Liddle, M. J., 1986. — The biology of Australian weeds. 15. *Xanthium occidentale* Bertol. complex and *Xanthium spinosum* L. *Journal of the Australian Institute of Agricultural Science* 52: 191-221.

Jones, R. W., Lanini, W. T., and Hancock, J. G., 1988. — Plant growth response to the phytotoxin viridiol produced by the fungus *Gliocladium virens*. *Weed Science* 36: 683-687.

Julien, M. H., Broadbent, J. E., and Matthews, N. C., 1979. — Effects of *Puccinia xanthii* on *Xanthium strumarium* [Compositae]. *Entomophaga* 24: 29-34.

Kochman, J. K., 1980. — Noogoora burr rust (*Puccinia xanthii*) on sunflower. *In:* Jessop, R., and Mowatt, P. J. (eds.). *Fourth Australian Sunflower Workshop, Shepparton, Victoria*. pp. 8-10. Australian Sunflower Association.

MARTIN, R. J., and CARNAHAN, J. A., 1983. — The effect of field storage and laboratory conditions on germination of five *Xanthium* species. *Australian Journal of Agricultural Research* 34: 249-260.

MCKEEN, C. D., and THORPE, H. J., 1973. — Pathogenic species of *Verticillium* in horticultural crops and weeds in southwestern Ontario. *Canadian Journal of Plant Science* 53: 615-622.

MCMILLAN, C., 1975. — The *Xanthium strumarium* complexes in Australia. *Australian Journal of Botany* 23: 173-192.

MCRAE, C. F., and AULD, B. A., 1988. — The influence of environmental factors on anthracnose of *Xanthium spinosum*. *Phytopathology* 78: 1182-1186.

MORIN, L., AULD, B. A., and BROWN, J. F., 1993. — Host-range of *Puccinia xanthii* and post-penetration development on *Xanthium occidentale*. *Canadian Journal of Botany*. In press.

MORIN, L., BROWN, J. F., and AULD, B. A., 1992. — Effects of environmental factors on teliospore germination, basidiospore formation and infection of *Xanthium occidentale* by *Puccinia xanthii*. *Phytopathology* 82: 1443-1447.

MUIR, D. B., 1988. — Preservation of fungi in sterile physiological saline. *Australian Microbiologist* 9: 358-359.

MUIRHEAD, I. F., 1981. — The role of appressorial dormancy in latent infection. *In:* BLAKEMAN, J. P. (ed.). *Microbial Ecology of the Phylloplane*. pp. 155-167. Academic Press, London, U.K.

NEHL, D. B., 1990. — *The Potential of Alternaria zinniae M. B. Ellis as a Mycoherbicide to Control Xanthium Weeds in Cotton*. B.Sc. Honours thesis, Department of Botany, University of New England, Armidale, NSW, Australia.

NIKANDROW, A., WEIDEMANN, G. J., and AULD, B. A., 1990. — Incidence and pathogenicity of *Colletotrichum orbiculare* and a *Phomopsis* sp. on *Xanthium* spp. *Plant Disease* 74: 796-799.

PAUL, N. D., and AYRES, P. G., 1990. — Effects of interactions between nutrient supply and rust infection of *Senecio vulgaris* L. on competition with *Capsella bursa-pastoris* (L.) Medic. *New Phytologist* 114: 667-674.

PHILLIPS, A. J. L., 1992. — Some common weed species as alternative hosts for *Sclerotinia sclerotiorum*. *Phytophylactica* 24: 207-210.

RAMSELL, J., and PAUL, N. D., 1990. — Preferential grazing by molluscs of plants infected by rust fungi. *Oikos* 58: 145-150.

ROY, K. W., 1982. — Seedling diseases caused in soybean by species of *Colletotrichum* and *Glomerella*. *Phytopathology* 72: 1093-1096.

SANDS, D. C., FORD, E. J., and MILLER, V., 1990. — Genetic manipulation of broad host-range fungi for biological control of weeds. *Weed Technology* 4: 471-474.

TEMPLETON, G. E., TEBEEST, D. O., and SMITH, Jr., R. J., 1979. — Biological weed control with mycoherbicides. *Annual Review of Phytopathology* 17: 301-310.

TERVET, I. W., and CHERRY, E., 1950. — A simple device for collection of fungus spores. *Plant Disease Reporter* 34: 238.

TOMIYAMA, K., DOKE, N., NOZUE, M., and ISHIGURI, Y., 1979. — The hypersensitive response of resistant plants. *In:* DALY, J. M., and URITANI, I. (eds.). *Recognition and Specificity in Plant Host-Parasite Interactions*. pp. 69-84. Japan Scientific Societies Press, Tokyo.

WALKER, J., NIKANDROW, A., and MILLAR, G. D., 1991. — Species of *Colletotrichum* on *Xanthium* (Asteraceae) with comments on some taxonomic and nomenclatural problems in *Colletotrichum*. *Mycological Research* 95: 1175-1193.

YAMADA, M., TAKAHASHI, K., TAKAHASHI, H., and TANAKA, T., 1973. — Studies on alternate host, *Thalictrum thunbergii* DC., as a (sic) origin of physiologic races of wheat leaf rust, *Puccinia recondita* Roberge ex Desm. f. sp. *tritici* in Japan. *Report of the Tottori Mycological Institute (Japan)* 10: 283-302.